Report for 2004CA90B: Hydrological regimes, pond morphology, habitat use: predicting the impact of an emerging aquatic pathogen

- Other Publications:
    - PUBLICATIONS FROM PRIOR PROJECTS. Please provide citations to publications attributable to projects completed in prior years and not included in this year's annual report. Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of Batrachochytrium dendrobatidis within and between amphibian life stages, Diseases of Aquatic Organisms, 61, 75-83. Rachowicz, L. J., J.-M. Hero, R. A. Alford, J. W. Taylor, J. A. T. Morgan, V. T. Vredenburg, J. P. Collins, and C. J. Briggs. (In Press) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife, Conservation Biology. PUBLICATIONS & CITATION FORMAT: List all reports, in the following format, published during the reporting period resulting from work supported by your project funding and by supplemental grants during the reporting period. Briggs, C.J, Vredenburg, V.T., Knapp, R.A., and L. J. Rachowicz. (In Press) Investigating the population-level effects of chytridiomycosis, an emerging infectious disease of amphibians, Ecology. Rachowicz, L. J., R. A. Knapp, J. A. T. Morgan, M. J. Stice, V. T. Vredenburg, J. M. Parker, and C. J. Briggs (In Review) Emerging infectious disease as a proximate cause of amphibian mass mortality in Rana muscosa populations, Submitted to Ecology. Rachowicz, L. J., 2005, Transmission of an emerging infectious disease in a declining amphibian species: Batrachochytrium dendrobatidis in the mountain yellow-legged frog (Rana muscosa) " Ph.D. Dissertation," Department of Integrative Biology, University of California, Berkeley, California, 210 pages.

Report Follows

RESEARCH PROGRAM:

Project Summary

Declines in amphibian populations have been reported throughout the world in recent years. A number of factors have contributed to these population declines, including disease, introduce species, habitat loss and alteration, and climate change. Chytridiomycosis is a potentially fatal disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis*, which has appeared recently in the aquatic habitats of California and throughout the world. In portions of the Sierra Nevada mountains of California, the disease is causing rapid die-offs of mountain yellow-legged frogs, *Rana muscosa*, a threatened native frog species. In other areas of the Sierra, infected populations of *R. muscosa* appear to be persisting with the fungus. In this study we are investigating why the fungal pathogen is having different outcomes on frog populations in different California watersheds.

The main hypothesis that we are investigating is that differences in the pond morphology and topography of the landscape in different areas result in the frogs using the habitat differently at the different sites, altering their risk of acquiring and succumbing to the disease. Frogs that spend most of their time aggregated in the main lakes and ponds at each site, and in colder temperature habitats, are at greater risk from the disease. Frog die-offs due to the disease in the Sierra are occurring mainly in areas consisting of deep lakes surrounded by granite bedrock, where the adult frogs spend the majority of their time in the lakes. The sites at which the frogs are persisting with the fungus include extensive marsh and stream areas with emergent vegetation, in addition to lakes. At these sites the frogs are not confined to isolated lakes, and may be able to escape from areas with high concentrations of zoospores (the infectious stage of the fungus by which the disease is spread). We are also investigating alternative hypotheses that differences in the transmission, infectivity, and/or virulence of the fungal strains, or differences in susceptibility of the frog genotypes at the different types of sites, are leading to the observed differences in the impact of the fungal pathogen.

Field Surveys: During the summer of 2004 we conducted detailed surveys at sites in the Sierra Nevada mountains experiencing *R. muscosa* die-offs due to chytridiomycosis, and sites with *B. dendrobatidis* present with persistent *R. muscosa* populations. We used a newly-developed real-time PCR (quantitative PCR) protocol to determine the infection status (presence/absence of *B. dendrobatidis*) and infection level (fungal loads) of *R. muscosa* individuals. This protocol involves non-destructive swabbing of the frog skin, and therefore can be used repeatedly on the same frog individuals. We performed repeated surveys at the field site over the course of summer 2004. At each site we quantified the abundance and stage-structure frog populations, the infection status of tadpoles and adults, documented the presence and abundance of other potential hosts for the disease, and quantified the habitat characteristics. At sites where marking was feasible (i.e. frog population densities were not too high) we marked adult *R. muscosa* using PIT tags (passive integrated transponder), and recaptured and re-swabbed the same individuals over the course of the summer. We found that infected frogs at die-off sites carried very high fungal loads, while at persistent sites the infected frogs were experiencing only low to moderate infections. Interestingly, at the persistent infected sites, some adult *R. muscosa* were found to lose the infection between the start and end of the summer. In the high Sierra, *R. muscosa* have a very short summer season in which they are active, with ice-free days spanning from mid-June through late September. A great deal of mortality in *R. muscosa* occurs during the long overwintering period. Therefore, we will be repeating these surveys in the summer of 2005 to determine if infected *R. muscosa* individuals at the persistent sites are able to survive over the winter.

Laboratory Experiments: From winter through summer 2004 we conducted a laboratory experiment to investigate the ecological differences in fungal pathogen strains collected from infected frogs in die-off and persistent sites. We experimentally inoculated uninfected tadpoles that had been raised from eggs in the laboratory with a known amount of zoospores of a fungal strain from either a die-off site or a persistent site. Each week we inspected the tadpoles for signs of infection (to determine differences in transmission of the different strains), and measured the rate of release of infectious zoospores (to determine differences in infectivity of infected tadpoles). We followed the tadpoles through metamorphosis to record differences in survival of the individuals exposed to the different strains. In the experiment we found that transmission of the fungus to tadpoles requires very high doses of zoospores, but we measured no differences between the fungal strains in transmission, virulence, infectivity, or tadpole survival. Thus, so far we have found no evidence that differences in fungal strains are responsible for the different population-level impacts of the disease at the different sites. We are in the process of repeating this experiment using post-metamorphic *R. muscosa*, which are more susceptible than tadpoles to *B. dendrobatidis*.

Antimicrobial peptides released from the skin of frogs are part of their innate immune response, and some peptides have been shown to be effective at killing *B. dendrobatidis* in laboratory cultures. From fall 2004 through spring 2005, we conducted an experiment to determine if the antimicrobial peptides that are released from *R. muscosa* are effective in protecting *R. muscosa* from *B. dendrobatidis*. Differences in the antimicrobial peptide response might help explain the different outcomes of the fungal pathogen observed in different parts of the Sierra Nevada mountains. Through this experiment we also quantified the dose-response curve of *R. muscosa* to *B. dendrobatidis*. *R. muscosa* subadults were exposed to specific quantified doses of *B. dendrobatidis* zoospores, and monitored to determine the fraction that became infected, and the fate of the infected individuals. In some treatments, the antimicrobial peptides were removed from the frogs prior to exposure to *B. dendrobatidis*. We found that *R. muscosa* individuals increased their peptide production following exposure to *B. dendrobatidis*. It was predicted that if antimicrobial peptides served to defend the frogs against the disease, then individuals from which peptides had been removed prior to exposure would be more likely to become infected. However, we found absolutely no difference between the fractions of individuals that became after peptide removal versus those that were exposed to the same dose without prior peptide removal. This suggested that the antimicrobial peptides are not sufficient to protect *R. muscosa* from chytridiomycosis in even a simple experimental situation.

INFORMATION TRANSFER PROGRAM:

None

STUDENT SUPPORT:

Undergraduate student Eleanore Sternberg was supported in part on the Water resources grant.

Graduate students: Lara Rachowicz and Mary Stice were each supported for part of the year on the Water Resources grant.

	Total Project Funding		Supplemental Awards	Total
	Federal Funding	State Funding		
Undergrad.	0.5	0.5	0	1
Masters	0	0	0	0
PhD.	1	1	0	2
Post-Doc.	0	0	0	0
Total	1.5	1.5	0	3

NOTABLE ACHIEVEMENTS AND AWARDS:

No awards. Notable achievements include the key results described above.

PUBLICATIONS FROM PRIOR PROJECTS. Please provide citations to publications attributable to projects completed in prior years and not included in this year's annual report.

Rachowicz, L. J., and V. T. Vredenburg. 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages, Diseases of Aquatic Organisms, 61, 75-83.

Rachowicz, L. J., J.-M. Hero, R. A. Alford, J. W. Taylor, J. A. T. Morgan, V. T. Vredenburg, J. P. Collins, and C. J. Briggs. (In Press) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife, Conservation Biology.

PUBLICATIONS & CITATION FORMAT: List all reports, in the following format, published during the reporting period resulting from work supported by your project funding and by supplemental grants during the reporting period.

Briggs, C.J, Vredenburg, V.T., Knapp, R.A., and L. J. Rachowicz. (In Press) Investigating the population-level effects of chytridiomycosis, an emerging infectious disease of amphibians, Ecology.

Rachowicz, L. J., R. A. Knapp, J. A. T. Morgan, M. J. Stice, V. T. Vredenburg, J. M. Parker, and C. J. Briggs (In Review) Emerging infectious disease as a proximate cause of amphibian mass mortality in *Rana muscosa* populations, Submitted to Ecology.

Rachowicz, L. J., 2005, Transmission of an emerging infectious disease in a declining amphibian species: *Batrachochytrium dendrobatidis* in the mountain yellow-legged frog (*Rana muscosa*) " Ph.D. Dissertation," Department of Integrative Biology, University of California, Berkeley, California, 210 pages.